

Standard Operating Procedures for the Collection and Analysis of Algae

June 2016 (Version 2.0)



Prepared by:

NORTH CAROLINA DEPARTMENT OF ENVIRONMENTAL QUALITY



Water Resources
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Division of Water Resources
Water Sciences Section
Ecosystems Branch

This report has been approved for release by:

Steven Kroeger July 5, 2016
Steven Kroeger, Supervisor, Ecosystems Branch Date

Cyndi Karoly July 7, 2016
Cyndi Karoly, Chief, Water Sciences Section Date

This is not a regulatory document. It is intended for use as a guide to procedures performed by the Water Sciences Section and is considered a working document that may have revisions and additions.

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ACRONYMS AND ABBREVIATIONS

BV (or bv)	biovolume
Bv ref	biovolume reference
ccm	cubic centimeter
cm	centimeter
DO	dissolved oxygen
DWR	Division of Water Resources
EB	Ecosystems Branch
HAB	harmful algal bloom
HUC	hydrologic unit code
ISB	Intensive Survey Branch
m	meter
µg	microgram
µS/cm	microsiemens/centimeter
mL	milliliter
mm	millimeter
NADED	North American Diatom Ecological Database
NCDENR	North Carolina Department of Environment and Natural Resources
NCDEQ	North Carolina Department of Environmental Quality
ppt	parts per thousand
QA/QC	Quality Assurance/Quality Control
SDS	Safety Data Sheet
SOP	standard operating procedure
SU	standard units
TSN	taxonomic serial number
USEPA	United States Environmental Protection Agency
WSS	Water Sciences Section

1.0 INTRODUCTION AND PURPOSE

This manual describes the standard operating procedures (SOP) for the sampling, fixation, preservation, identification and enumeration of algae in North Carolina surface waters, and procedures for quality control. This SOP is used by the NC Division of Water Resources (DWR) staff within the Water Sciences Section (WSS). Consistency in sample collection and data analysis are the cornerstones for evaluating algal assemblages. Analytical procedures provided in this manual follow methods outlined in the Standard Methods for the Examination of Water and Wastewater – 20th Edition (APHA 1998).

Algae are a diverse group of multicellular organisms that can be submerged, floating, or emergent, that contain chlorophyll-*a* and have unicellular reproductive structures. They can positively affect water quality by storing and removing nutrients from aquatic systems. They also provide food and shelter for fish and invertebrates. However, algae are responsive to the physical and chemical conditions in the aquatic environment and may negatively affect water quality when excessive growths impact aquatic systems by clogging waterways and hampering recreation. Their rapid reproduction in response to nutrients can cause prolific growths, known as blooms. Blooms often occur when environmental conditions are favorable to a particular taxon or group of taxa, such as an extended photoperiod during summer, excessive nutrients, and slow or non-flowing waters. Algal blooms can occur as overgrowths throughout the water column (phytoplankton blooms), in particular sections of the water column (surface and metalimnetic blooms), or in dense filamentous mats. Algal blooms can produce changes in water chemistry, most notably pH and dissolved oxygen (DO). Excessive algal biomass may threaten fish health during the microbial decomposition of algal cells that depletes levels of available DO.

Algae may be a concern in drinking water supplies. Some harmful algal blooms (HABs), such as *Microcystis*, may produce toxins and have been linked to deaths of livestock and domestic pets in other states. Some species of algae may also cause taste and odor problems, water discoloration, or form large mats that can interfere with recreational activities and commercial boating.

Algae are identified to the lowest taxonomic level possible, which quantifies problematic species and helps discern the causes of taste and odor reports and filamentous mats. The WSS does not have the capacity to test samples for algal toxins; therefore, it works closely with the Department of Health and Human Services – Division of Public Health and/or researchers at the following institutions when potentially toxic algae are an issue:

- University of North Carolina at Wilmington
- National Ocean Service/National Oceanic and Atmospheric Administration in Beaufort, North Carolina
- UNC-Chapel Hill Institute of Marine Sciences in Morehead City, North Carolina

This manual is reviewed and revised as needs dictate. All current employees and new employees within the Algal Lab of the EB will be provided with this manual to serve as a guideline for activities, methods, and procedures. SOPs and quality assurance/quality control (QA/QC) procedures in this manual will be the basis for algal assemblage assessments performed by WSS in the waters of North Carolina and the subsequent data provided in memos and reports. Deviations from these procedures for unusual sampling situations shall be documented in an appropriate report or memo.

2.0 SAMPLE ANALYSIS CONSIDERATIONS

2.1 Summary of the Method

Two types of algal evaluations are conducted by the WSS:

- Routine evaluations are conducted at selected stations to assess changes in algal assemblages over time (i.e., year-to-year, month-to-month).
- Episodic evaluations are conducted to document algal activity at a particular time or during a particular event. These events include algal blooms, fish kill investigations, and nuisance algal growths.

2.2 Health and Safety

The WSS follows internal safety procedures as well as those outlined in [Standard Operating Procedures Manual: Physical and Chemical Monitoring](#) (NCDENR 2013), and Fish Kill Response Procedures (NCDENR 2015). For a copy of the WSS Safety Procedures Guidelines, contact the WSS Safety Officer.

The majority of work performed by the Algal Lab group occurs in the laboratory environment. As such, basic safe handling and laboratory practices are employed during sample handling. All broken microscope slides, cover slips, and broken glass must be disposed of in a labeled glass disposal container. Subsamples being prepared from a sample that may represent a health risk should be handled with gloves and safety glasses, and poured in a fume hood.

Lugol's solution is a chemical mixture that is used for preservation and fixation of algae samples. Caution must be used while handling, preparing, and storing Lugol's solution as it can irritate the skin, nose, and throat, as well as stain skin and clothes. Lugol's solution must be stored in a fume hood or well-ventilated area. Safety Data Sheets (SDS) on this solution must be kept on file. To obtain a copy of the SDS, contact the WSS Safety Officer or the vendor that provided the Lugol's solution.

Periodically, staff from the Algal Lab are required to perform primary sample collection. In those cases, algal samples can be collected from locations throughout North Carolina, and at times and places where medical facilities may not be readily available. The WSS has a safety committee that is responsible for maintenance and development of current safety procedures and checklists to which all personnel are required to adhere. All employees must follow these safety precautions when using equipment and hazardous materials.

2.3 Personnel Qualifications

An experienced algal taxonomist must conduct all taxa identifications and enumerations.

Analytical procedures outlined in this document require expertise in microscopy and taxonomy to:

- document problematic areas;
- identify problematic taxa and their distribution;
- help investigate possible causes of fish kills;
- help investigate taste and odor problems in drinking water supplies; and
- address concerns about discolored waters, surface films, and unusual growths.

WSS also complies with all United States Environmental Protection Agency (USEPA) requirements regarding competency of field staff, laboratory procedures, and other quality assurance measures as described in the approved Quality Management Plan (NCDENR 2015).

2.4 Microscopy

Microscopes are needed to perform algal analyses. Measurements of an alga or a field of view are made with a reticula (scale) or grid that has been placed within the ocular of the microscope. Determining the distance between the lines in the reticule or the area of a grid at various magnifications requires a micrometer slide.

2.4.1 Microscopes

The WSS Algal Lab uses two styles of microscopes:

- A Leitz Laborlux S compound microscope with 10X oculars; 10X, 25X, 40X, 63X, and 100X (oil immersion) objectives (maximum magnification: 1000X) equipped with an attached three module epifluorescence illuminator with filters; and an HBO 100 watt mercury bulb.
- Two Leitz Diavert inverted microscopes: (1) one with 10X oculars and 4X, 10X, 20X, and 32X objectives (maximum magnification: 320X); and (2) one with 15X oculars and the same objectives (maximum magnification: 480X). Both inverted microscopes are illuminated by Leitz 100W transformers.

2.4.2 Microscope Maintenance

Microscopes must be maintained and serviced to provide optimum visual clarity. Microscopes are covered by a lint free cloth or plastic when not in use. Objectives and oculars are cleaned using an approved lens cleaner, non-abrasive cotton swabs, and lens paper. A certified technician services the microscopes annually.

2.4.3 Microscope Calibration and Standardization

All reticules (the scale inside the ocular used for measurement and area determinations) must be calibrated periodically for all magnifications to ensure quality measurements.

2.4.3.1 Determining the Grid Area on a Whipple Disc:

1. Place a micrometer slide (a slide with a small ruler on it divided into microns) on the stage and under the lowest power objective.
2. Align the edge of the grid with the first line on the micrometer slide.
3. Count the number of lines within the grid to determine the length of the total grid and individual grid divisions.
4. Record this measurement and place on or near each microscope for quick reference (Table 1).
5. Repeat this procedure for all objectives.

Since the grid on a Whipple disc is square, multiply the length times the width to get the area. This is the measurement (area) used in the calculation to determine cell and unit density outlined in Section 5.3 of this SOP.

Table 1. Example of Measurements and Areas of a Whipple Disc Grid

Objective	Grid width (µm)	Large grid divisions (µm)	Small grid divisions (µm)	Area (cm ²)
10X	900	90	20	0.020250
20X	450	45	10	0.008100
32X	280	28	6	0.000784

NOTE: µm (micrometers) are converted to cm² for use in the density calculation by multiplying by 1000

2.4.3.2 Determining the Distance Between Lines on a Reticule:

1. Place a micrometer slide (a slide with a small ruler on it divided into microns) on the stage and under the lowest power objective.
2. Align the first line of the micrometer slide to the first line of the reticule.
3. Count the number of lines within the reticule to determine the length and the individual divisions of the reticule.
4. Record this measurement and place near or on each microscope for quick reference (Table 2).
5. Repeat this procedure for all objectives.

Table 2. Example of Ocular Reticule Grid Divisions for the Leitz Laborlux S Compound Microscope

Objective	Width of gradations (μm)
10X	10.0
25X	4.0
40X	2.5
63X	1.6
100X	1.0

3.0 SAMPLE PROCESSING PROCEDURE

3.1 Sample Metadata

Sample metadata includes: 1) sampling methodology; 2) sample type; and 3) associated environmental parameters collected.

3.1.1 Sampling Methods

There are three sampling methods that can be used to collect algae. The sample method must be noted on the label, container, field sheet, and episodic evaluation forms.

3.1.1.1 Photic Zone Samples

These are integrated depth profiles that must be collected with a Lab-line Poly Pro Water Sampler® (Lab-line®). A Secchi disc is first used to determine depth of visibility in the water column; the photic zone is the depth to which ambient light penetrates and is defined as twice the Secchi depth. A composite sample is collected by lowering the Lab-line® through the photic zone at a constant speed. It is preferred over other sample collection methods because it provides a sample of the water where phytoplankton (algae) may be present in the water column (approximately 2x Secchi depth). This method should only be performed in waters that are deeper than one meter.

3.1.1.2 Grab Samples

These (also known as point samples) are collected by inverting a container, placing it below the water surface, then turning it upright and allowing it to fill. Grab samples can also be taken from a bridge or dock by quickly lowering a container 6 inches (15 cm) below the surface. This method can be used during a fish kill and when a lab-line is not available or practical.

3.1.1.3 Scoop Samples

Scoops are surface skims, dips, scrapings, or any other method of collecting the sample. This method should be used when only the identification of the algae is important.

3.1.2 Sample Type

There are three sample types:

- Filamentous algae (i.e., algae in long strands that can be picked up by hand);
- Periphyton (i.e., algae growing on rock, soil, or sand); and
- Phytoplankton (i.e., algae growing in the water column).

3.1.3 Environmental Conditions

Physical and chemical conditions (temperature, DO, pH, Secchi depth, etc.) must be recorded and collected according to methods outlined in the Intensive Survey Branch (ISB) SOP: Physical and Chemical Monitoring (NCDENR 2013).

3.2 Quantifying Algal Blooms

Three measures may be used to quantify algal blooms. These measures are used independently as described below:

3.2.1 Chlorophyll-*a* Measurements

The WSS determines the level of chlorophyll-*a* with a fluorometer, according to United States Environmental Protection Agency (USEPA) Standard Method 445.0 (Arar & Collins, 1997). These estimates

of phytoplankton biovolume are used as the primary measure in phytoplankton bloom determination. Chlorophyll-*a* levels that exceed 15 µg/L in trout waters or 40 µg/L in other waters are a violation of the State water quality standard (Rule 15A NCAC 02B.0211¹).

3.2.2 Unit Density

Algal units are growth forms, such as colonies, filaments, or unicellular. Unit density (expressed as units/mL) is determined by counting the number of algal units in a subsample.

3.2.3 Cell Density

Cell density, expressed as cells/mL, is the number of algal cells in a growth form, such as a colony, filament, or unicellular, and is determined by counting the number of cells in ten growth forms of a particular alga encountered during the analysis. These ten counts are averaged and multiplied by the total number of units found (Section 3.2.3) in order to estimate algal cell density.

3.2.4 Biovolume

Biovolume (expressed as millimeters [mm]³/meters[m]³) is an estimate of the volume of phytoplankton within a cubic meter of water. It is determined by counting the number of algal cells in a subsample and multiplying that number by a reference cell biovolume. The reference biovolume (*bv_{ref}*) is an estimate based upon measurements made on cells of the taxon from previous samples. The accuracy of this measurement varies from season to season and from taxon to taxon.

3.3 Algae and Fish Kill Investigations

Phytoplankton samples collected as part of fish kill investigations are analyzed for species composition and density to help discern whether algae were a factor in the event. Detailed information on fish kills, precautions, and collection procedures can be found in Fish Kill Events: <http://www.ncnhp.org/web/wq/ess/fishkills>.

3.4 Nuisance Growths

Nuisance growths are those that people find objectionable, those that may degrade water quality with taste and odor issues, and/or those that impact designated use(s) with large filamentous algal mats. Most nuisance growth samples are qualitatively analyzed (identification only) for taxa composition. Taste and odor samples may be quantitatively analyzed (identification and enumeration) for density and biovolume to determine the extent and potential duration of the problem.

3.5 Episodic Events

Two field evaluation forms have been developed to record required data for episodic events (Appendix A):

- Sample Collection and Field Evaluation Form
- Fish Kill Investigation Form

These forms are used to document environmental conditions and serve as event records. Samples submitted as phytoplankton blooms or as part of fish kill investigations are given priority. Evaluation forms should include:

- Sample ID
- Date/Time collected
- Location (including waterbody, latitude/longitude, station, city, county, basin, and hydrologic unit code (HUC) information)
- Sample collection method

¹<http://reports.oah.state.nc.us/ncac/title%2015a%20-%20environmental%20quality/chapter%2002%20-%20environmental%20management/subchapter%20b/15a%20ncac%2002b%20.0211.pdf>

- Sample type
- Analytical method (quantitative or qualitative)
- Collector's name
- Site photograph

Data recorded on evaluation forms should include:

- DO (mg/L and % saturation)
- Salinity (parts per thousand [ppt])
- pH (standard units – SU)
- Secchi depth (m)
- Water temperature (°C)
- Nutrients sampled
- Air temperature (°C)
- Chlorophyll-*a* (µg/L) sampled
- Conductivity (µS/cm)
- Visual description or site photograph

If possible, chlorophyll-*a* samples should be taken during phytoplankton blooms, fish kill investigations, and taste and odor evaluations. Episodic evaluation forms can be submitted to the WSS electronically (mark.vanderborgh@ncdenr.gov or elizabeth.fensin@ncdenr.gov), faxed (919-743-8517), or sent by interoffice mail (1623 Mail Service Center) along with the sample.

3.6 Sample Handling

Water chemistry samples must be collected, preserved, and shipped in accordance with WSS requirements ² and the Intensive Survey Branch [Standard Operating Procedures Manual: Physical and Chemical Monitoring](#) (NCDENR 2013).

3.7 Sample Containers

All photic zone and grab samples should be collected in a 500 mL plastic bottle. Scoop samples are not restricted to any particular container as long as they can be sealed and are clearly labeled. Macroalgae should be wrapped in a wet paper towel or newspaper and placed in a labeled, sealed plastic bag.

3.8 Sample Preservation

Adding a preservative is required to maintain algal cell structure, reduce microbial decomposition, halt grazing activity by zooplankton, and stop cell division. All samples should be kept cool and out of direct sunlight.

The recommended preservative for phytoplankton and scraped periphyton samples is a modified Lugol's solution (Vollenweider 1974). Lugol's solution should remain chemically stable for up to 5 years when kept in a dark container in a cool dry place. Lugol's solution can be obtained by contacting the WSS or prepared by following the directions outlined in Appendix B.

Approximately 3 to 4 mL of Lugol's solution is required for 500 mL of sample. The exact quantity will depend on the algal density and particulate matter in the sample. A well-preserved sample should be a dark straw or tea-like color.

3.9 Sample Quantity

The quantity of sample required (Table 3) varies according to the evaluation (episodic or routine), sample type, and sample method. Episodic and routine phytoplankton evaluations require one 500 mL preserved photic or grab

²<https://ncdenr.s3.amazonaws.com/s3fs-public/Water%20Quality/Chemistry%20Lab/Operations/Staff%20Resources/PreservationHoldTime-SurfaceWaterSamples-July232015-WSSChemLab-FINAL.pdf>

sample. Episodic phytoplankton evaluations require one 500 mL preserved and one 500 mL unpreserved photic, grab, or scoop sample. Episodic periphyton evaluations require 50 mL of rock (or other substrate) scrapings (preferably a mix from several areas) or two small (< 4 inch) rocks. Episodic filamentous algae evaluations require two to three small (golf ball-sized) clumps, preferably from different areas.

Table 3. Sample Quantity and Preservative Requirements

Sample Type	Preserved	Unpreserved
Phytoplankton - routine sample	500 mL	NA
Phytoplankton - episodic sample	500 mL	500 mL
Periphyton	500 mL of wash from scraping	2 small 4-inch rocks or sticks
Filamentous algae	NA	2 or 3 golf ball sized clumps

3.10 Sample Labeling

All algal samples must be clearly labeled with a sample tag or a permanent marker. The tag must include:

- Waterbody
- Station Code/Number
- Date/Time Collected
- Sample ID
- Sample Collection Method
- Collector
- Preservative (if applicable)

Note: "FISH KILL" must be written on the label of a sample collected during a fish kill investigation.

3.11 Sample Shipping

Algal and chemistry samples must be kept on ice and either brought to the WSS or shipped by Interoffice Mail as soon as possible to:

Division of Water Resources – WSS
 ATTN: Ecosystems Branch
 1623 Mail Service Center
 4405 Reedy Creek Road
 Raleigh, NC 27607

3.12 Sample Receiving

When collecting water chemistry samples, [Sample Collection and Submittal Forms \(DM1\)](#) must be completed on all samples before submittal. Contact the [WSS Microbiology and Inorganic Chemistry Branch](#) for information required for sample processing.

All samples stored at 4405 Reedy Creek Road, with the exception of unpreserved estuarine fish kill samples, are kept refrigerated at 4 °C until received by Algal Lab staff. Samples are collected from 4405 Reedy Creek Road several times a week. Algal Lab staff should be notified by field investigators when priority samples, such as fish kills and algal blooms, are being sent. For samples that might be used in legal proceedings, Chain-of-Custody procedures must be followed as outlined in the Intensive Survey Branch [Standard Operating Procedures Manual: Physical and Chemical Monitoring](#) (NCDENR 2013).

3.13 Sample Storage

3.13.1 Short-term sample handling

Preserved algal samples should be stored in a dark, dry, cool place prior to analysis.

3.13.2 Long-term sample handling

Phytoplankton samples that have been processed and analyzed are archived in the WSS lab until the study is completed. For example, basinwide samples are discarded prior to the next round of sampling. Estuarine samples are kept for at least one month in case any questions about particular samples arise. All unpreserved samples are discarded after analysis.

3.14 Sample Data Management

Samples analyzed by Algal Lab staff are given a unique tracking number when they are entered into a Microsoft Access® database. The nine-digit number is automatically generated with the first five numbers being the line number of the database Entry Log Table followed by the four-digit year. For example, the sample id number 10245-2015 was given to the 10245th sample entered into the database during the year 2015.

3.15 Sample Data Management Fields

The following sample data are entered into a database:

- Date/time collected
- Waterbody
- Station code/number
- Station description
- Latitude/longitude
- County
- EcoType (Estuary, River/Stream, Lake/Reservoir, Other)
- Basin
- Sample type (phyto-, peri-, fila-)
- Collector
- ID method (quan/qual)
- Sample method (Photic, Grab, Scoop)
- Assessment type (Episodic, Routine)
- Episode description (Fishkill, Bloom, Other)
- Date received
- Entered by
- Chemistry lab number (if available)
- Physical data (if available)
- Chemical data (if available)
- HUC

4.0 LABORATORY TECHNIQUES AND ANALYSIS

4.1 Sample Analysis

Microscopes are necessary to conduct algal/phytoplankton analyses, which can be quantitative or qualitative.

A quantitative analysis identifies taxa to the lowest taxonomic level possible and enumerates a sample providing information on:

- algal density
- estimates of biovolume
- assemblage composition
- dominant taxa

A qualitative analysis identifies taxa to the lowest taxonomic level possible and provides information on:

- assemblage composition
- dominant taxa

The sample analysis process includes:

- preparation of a subsample
- a visual check of the subsample for density and distribution (quantitative analysis only)
- recording required data about the sample (e.g., location) and subsample (e.g., taxon) on a bench sheet

4.2 Subsample preparation

Two types of subsample preparation techniques are used for algal analysis:

4.2.1 Wet mount preparation

Wet mounts are used on compound microscopes. They should be used when high magnification (> 400X) is needed for taxa identification and cell or unit density is not important. Prepare a wet mount by placing a small aliquot (≈ 0.05 mL) of sample on a glass microscope slide or Palmer slide and then covering it with a coverslip.

4.2.2 Utermöhl chamber preparation

Utermöhl chambers are used on inverted microscopes. They are particularly useful for samples with a wide range of salinity because the salts remain in suspension. They must be used for all quantitative analyses. Utermöhl chambers have four parts:

- A threaded metal base.
- A threaded Plexiglas chamber that comes in 5 cubic centimeters (ccm) and 10 ccm sizes.
- A replaceable bottom coverslip.
- A circular glass top plate.

Note: 1 ccm = 1 cm³ = 1 mL.

Utermöhl chambers must be thoroughly cleaned before each use. To clean an Utermöhl chamber:

1. Rinse out the chamber with tap water.
2. Wash the inside of the chamber and both sides of the glass coverslip with a nonabrasive, non-residue soap and a cotton swab. Rinse out the chamber at least three times with distilled water.
3. Dry with a nonabrasive, no-lint paper towel.
4. Visually inspect the cleaned chamber to ensure that no oils or residues are remaining.

Cleaned chambers can be stored by placing them upside down on a clean surface.

Cracked or broken bottom coverslips must be replaced. To replace a bottom coverslip on an Utermöhl chamber:

1. Unscrew the chamber cylinder from the metal base.
2. Wash the chamber with a non-abrasive, non-residue soap, and water.
3. Dry the chamber with a non-abrasive, no-lint paper towel.
4. Apply a light coat of petroleum jelly on the threads of the cylinder.
5. Place a new coverslip in metal base.
6. Screw cylinder into ring.
7. Remove any excess petroleum jelly from chamber and coverslip with a non-abrasive, no-lint paper towel.
8. Wash the chamber.

To prepare a subsample using an Utermöhl chamber:

1. Bring the bottled sample to room temperature to equalize gas pressure to reduce air bubble formation after the subsample has been poured in the chamber.
2. Determine the size of chamber to use by evaluating the amount of particulates on the bottom of the sample container. Use a 5 ccm chamber for heavy (easily visible) particulates or a 10 ccm chamber for light (barely visible) particulates.
3. Check the bottom coverslip on the chamber for any particulates, oils, or films that would obscure the algae or affect an even distribution during settling.
4. Re-suspend the algae in the 500 mL polyethylene container by gently inverting it nine or ten times. Do not vigorously shake the sample container, as care must be taken to reduce aeration that can cause air bubble formation in the chambers. If samples have not been analyzed within a few weeks of collection, a spinner plate may be used to break up any clumping within the sample.
5. Slowly pour a subsample from the sample container into the chamber until it is completely filled and a convex meniscus forms at the top of the chamber.
6. Slide the circular glass plate onto the top of the chamber removing the convex meniscus and sealing the chamber.
7. Allow subsamples to settle for a minimum of 8 hours on a clean level surface.
8. The preparation may last up to a week without desiccation. A few drops of water may be added into settled samples to remove bubbles; however, care is required not to disturb the settled material. If more than a few drops of water are needed to remove bubbles, another subsample should be poured and allowed to settle. If deionized water is used in a sample it may disturb the ionic balance of a subsample.

4.3 Visual subsample density and distribution check

The quality of the analysis depends on the density and distribution of the subsample in the prepared Utermöhl chamber. A visual inspection must be performed on all Utermöhl chamber preparations for subsample density and random distribution. In general, the chamber should not have algae overlapping or obscuring the identification or enumeration of other algae. The algae should also be equally distributed throughout the Utermöhl chamber.

To check for proper density and uniform distribution:

1. Place the prepared Utermöhl chamber on the microscope stage.
2. Briefly scan the prepared Utermöhl chamber for distribution at low magnification ($\approx 100\times$).
3. The subsample should be evenly distributed throughout the chamber.
4. Non-evenly distributed subsamples or samples that are clumped together should not be quantitatively analyzed (either discard the sample or pour another subsample).
5. Adjust the microscope to a higher magnification ($\approx 320\times$).

6. The ideal density of algal units within the field of view is between 10 and 40 units.
 - a. Dense subsamples (> 40 units within a field of view at 320X) should be re-poured using a smaller chamber or diluted. Dilution can be done by extracting 50 mL of the sample, placing it into an unused polyethylene container and adding a known quantity of filtered (0.45 µm) water. It is important to use dilution water with a similar salinity to the sample as to maintain the osmotic balances of the algal cells.
 - b. Sparse subsamples should be re-poured using a larger chamber or concentrated. Concentration can be done by siphoning a known amount of water (i.e., ½) from a settled sample. Caution, this technique is prone to error. When siphoning, the nozzle must not touch the sides or get near the bottom.
 - c. Deionized water or other solution may be used to adjust salinity of samples collected from estuarine, marine, or brackish water environments.
 - d. Any alterations to the volume of the sample or the subsample must be recorded on the sample container and on the bench sheet.

4.4 Recording data

A quantitative analysis requires more detailed data to be recorded about a sample than a qualitative analysis. These data are required to calculate the density and biovolume of the algae in the sample. If these data are not known or unavailable, then the sample cannot be fully quantified. All data on the analysis must be recorded on an approved bench sheet.

4.5 Bench sheets

The bench sheets for quantitative or qualitative analysis are kept in accordance with North Carolina public records statutes. Data on the bench sheets must be written legibly so that others may read and understand notes, identifications, and counts. An example of the bench sheets can be found in Appendix C.

Bench sheet data must include:

- sample tracking number
- analyst name
- date the sample was analyzed
- taxa codes of the algae identified

Bench sheets used for quantitative analysis must also contain:

- quantity of the subsample analyzed
- area of the Utermöhl chamber analyzed
- number of algal cells in the area analyzed
- number of algal units in the area analyzed

The total number of cells and units must be calculated and recorded in the appropriate box on the bench sheet when a quantitative analysis is completed. These totals are used later after the data are entered to verify that the numbers of units and cells entered are equal to the number of units and cells on the bench sheet.

Qualitative analysis may require recording data on the number of cells and units of the taxa identified during the analysis. The number of cells and units can be used to determine the relative abundance of those taxa. Relative abundance is the abundance of a particular taxon in relationship to other taxa and is used to provide an estimate of what taxon is dominant in the sample.

4.6 Recording the number of algal cells

Algal cell counts are required to calculate algal biovolume in a sample. However, counting all the algal cells of all the algal units found during an analysis is impractical. Therefore, an average number of cells in a taxon's unit can be determined and then applied to the total number of units counted when the analysis is completed. This procedure can be performed before, during, or after the analysis using any style of microscope with the appropriate magnification.

To obtain an average number of cells in a taxon's unit:

1. Enumerate the number of cells in at least 10 units ($n > 10$) of the selected taxon.
2. Record the number of cells of each unit in the margin of the bench sheet.
3. When the analysis is completed, determine the average number of cells in a unit using the formula below.
4. Multiply the average number of cells per unit by the total number of units counted and round to the nearest whole number.
5. Record this number in the appropriate box on the bench sheet.

$$\frac{\text{Total Cells}}{\text{Total Units}} = \text{Average number of cells per unit}$$

Where:

- *Total Cells* is the total number of the particular taxon's cells that were counted to be averaged.
- *Total Units* is the total number of the particular taxon's units where cells were counted.

Example of obtaining an average number of cells in a unit.

- *Pseudanabaena* is a filamentous blue green alga.
- Ten filaments (units) in sample A were counted (Total units = 10).
- The numbers of cells in each of the 10 units were: 11, 8, 10, 2, 5, 6, 11, 4, 10, 8.
- The total number of cells counted was 75 (total cells = 75).
- The average number of cells per unit was 7.5 ($75/10 = 7.5$), rounding = 8.
- Thus, there were eight cells per unit (8 cells/unit) of *Pseudanabaena* in sample A.

4.7 Quantitative analysis

All quantitative analyses are performed on Leitz Diavert inverted microscopes as described in Section 2.4.1; a Whipple disc in the ocular; and an Utermöhl settling chamber filled with the subsample to be analyzed. A Whipple disc, described in Standard Methods, is inscribed with an accurately-ruled grid that is subdivided into 100 squares. A grid is defined as the total area enclosed within the most outside lines-the square perimeter of the Whipple disc; the size of the grid will vary based upon the level of magnification. It is the responsibility of the analyst to know the area of the grid he/she is using. (Refer to Section 2.4 to determine the area of the grid on a Whipple disc and other microscope calibration procedures.)

To identify and enumerate an algal assemblage in a sample:

1. Place a prepared Utermöhl chamber on the microscope stage.
2. Record sample data on a phytoplankton bench sheet (i.e., tracking code and size of chamber being used).
3. Randomly select an area to be analyzed.
4. Identify (to the lowest taxonomic unit possible) all taxa and the number of their cells and units within the entire Whipple disc area or touching the right and bottom grid lines.
5. Only count units that contain cytoplasm (i.e., disregard empty cells and disintegrating filaments or colonies).

6. Continue to analyze randomly selected discs until 100 units of the most abundant taxon is reached.³
7. Record the number of discs analyzed, the algae found, and the number of their cells and units on the bench sheet.
8. Tabulate the total number of units and cells for each taxon identified and record the totals into the space provided on the bench sheet.
9. Tabulate the total number of cells and units for the entire analysis and write it in the space provided on the bench sheet.
10. Enter bench sheet data into the database.

4.8 Qualitative analysis

Two types of qualitative analyses are performed:

- Identification only ('What is it?').
- Identification and enumeration for taxa dominance ('Which is the most common?').

These analyses are done by various means and not restricted to a particular microscope, slide preparation technique, or magnification.

4.8.1 Identification only

1. Place prepared slide or chamber on the microscope stage.
2. Record the sample data on a bench sheet (i.e., tracking code and date of analysis).
3. Identify the algae in the sample.
4. Record identifications on a bench sheet.
5. The bench sheet data are now ready to be entered into the database.

4.8.2 Dominant taxa analysis

1. Place prepared slide or chamber on the stage of microscope.
2. Record all sample data on a bench sheet (i.e., tracking code, date of analysis).
3. Identify the algae and count their number of cells and units in a field. (A field can be the grid on a Whipple disc or the complete field of view of the objective.)
4. Continue analyzing fields until at least 10 units of 10 taxa are reached.
5. Tabulate the total number of cells and units for each taxon.
6. Tabulate the total number of all cells and units of all taxa and record the total on the bottom of the bench sheet.
7. The bench sheet data are now ready to be entered into the database.
8. The dominant taxa can be defined either by greater number of units or by the largest biovolume depending upon the type of information needed.

³ Exceptions to Step #6 are: Sparse samples (i.e., few algal cells present) -- analyze at least 0.5% of the Utermöhl chamber (i.e., 40 grids at 300X magnification). Diverse samples with no dominant taxon -- analyze until a total of 300 units is reached.

5.0 DATA HANDLING AND INTERPRETATION

5.1 Taxonomic Reference Table

A taxonomic reference table of the algal taxa known to be present in North Carolina was developed by WSS staff and is utilized by the Algal Lab for taxonomic identification and is available upon request. This table provides a uniform structure for assigning taxonomic codes with scientific nomenclature. The taxa list is limited to phytoplankton at this time, but it is designed to incorporate other forms of algae in the future.

A taxonomic reference table requires periodic maintenance to update nomenclature and add new taxa.

5.1.1 Table Structure and Contents

5.1.1.1 Taxonomic Designation

The taxonomic designation of algae listed in the taxonomic reference table is based upon widely accepted botanical classification conventions as defined in two primary reference books: *Freshwater Algae of North America* (Wehr *et. al.* 2015) and *Identifying Marine Phytoplankton* (Tomas *et al.* 1997). Algal groups are listed instead of phyla or divisions because “algae” is a collective term that refers to a number of loosely-related organisms and not a formal classification convention. There are 10 algal groups included in the DWR algal database (Table 4).

Table 4. The Ten Algal Groups Listed in the Taxonomic Reference Table

Algal Group	Group Common Name	Algal Group Code
Bacillariophyceae	Diatoms	10
Chlorophyta	Greens	11
Chrysophyceae	Chrysomonads	12
Cryptophyta	Cryptomonads	13
Cyanobacteria	Blue greens	14
Ebriidea	Ebriidea*	15
Euglenophyta	Euglenoids	16
Prymnesiophyceae	Prymnesiophytes*	17
Pyrrhophyta	Dinoflagellates	18
Raphidophyceae	Raphidophytes*	19

* relatively small groups with no known common name

5.1.1.2 Taxonomic Reference Information

All taxa listed in the taxonomic reference table must be fully referenced to allow verification and review. Reference information includes: the scientific literature (e.g., reference book, journal article) that has the description of the taxon; the page number containing the taxon description; and the authority (person who originally described the taxon).

The majority of the nomenclature follows two primary reference books: *Freshwater Algae of North America* (Wehr *et. al.* 2015) and *Identifying Marine Phytoplankton* (Tomas *et al.* 1997). Secondary reference books, such as *A Manual of Fresh-Water Algae* (Whitford & Schumacher 1984) and *Studies on Brackish Water Phytoplankton* (Campbell 1973), are also used when they contain information not available in the primary references.

5.1.1.3 Taxonomic Serial Numbers

The taxonomic reference table contains two versions of taxonomic serial numbers (TSNs): both DWR and North American Diatom Ecological Database (NADED) numbers. These are also referred to as “reference codes” that are unique to a specific taxon. Codes are commonly used in place of scientific names to improve accuracy and efficiency in data entry, data storage, and data retrieval. An abbreviated version of the taxon’s scientific name (i.e., *Anacystis cyanea* = anac cya) was used previously by NCDWR staff. However, abbreviations become problematic when multiple names are spelled similarly or when new codes have to be created to accommodate changes in nomenclature. Therefore, TSNs were developed to avoid these problems. The TSNs are in the form of a 6-digit sequence. The first two digits designate the algal group, such as diatom or green algae. The third and fourth digits designate the genus. The fifth and sixth digits designate the species.

5.1.1.4 Assigning WSS Taxonomic Serial Numbers

The WSS uses the following steps to assign new TSNs:

1. Assign a TSN to a new taxon beginning with the algal group’s number (10 through 99). If the algal group has not been previously assigned a number, then use the next consecutive number after the last group number used.
2. Use the two-digit genus code if one exists (01 through 99). If the taxon is a new genus in the group, then use the next consecutive number after the last genus number used.
3. Use the next consecutive number after the last number used for a species in that genus (1 through 99). If the species has not been determined, then use double zeroes (00) to signify that the identity of the species is not known.

▪ **Example A.** The TSN for *Anabaena* spp.

- The first two digits, the number 14, designate that it is a blue green alga.
- The third and fourth digits, the number 01, designate that it is the genus *Anabaena*.
- The fifth and sixth digits, the number 00, designates that it is an undetermined species of *Anabaena*.
- The completed TSN for *Anabaena* spp. is 140100.

▪ **Example B.** The TSN for *Anabaena circinalis*.

- The first two digits, the number 14, designates that it is a blue green alga.
- The third and fourth digits, the number 01, designates that it is the genus *Anabaena*.
- The fifth and sixth digits, the number 02, designates that it is the species *A. circinalis*.
- The completed TSN for *A. circinalis* is 140102.

▪ **Example C.** Assigning a TSN to the new taxon *Anabaena planctonica*

- The first two digits, the number 14, designate that it is a blue green alga
- The third and fourth digits, the number 01, designate that it is the genus *Anabaena*.
- The fifth and sixth digits, 04 (the next consecutive number after the *A. flos-aquae*), designate that it is the species *A. planctonica*
- The completed TSN for *A. planctonica* is 140104

5.1.1.5 North American Diatom Ecological Database (NADED) Numbers

The North American Diatom Ecological Database numbers (NADEDs) are also listed in the taxonomic reference table. The NADED numbers were originally created for a database on diatoms at the Academy of Natural Sciences. The database was later expanded to include all forms of algae. The NADED numbers listed on the table have been created by the Academy of Natural Sciences of Philadelphia and are used to integrate WSS data with outside laboratories.

5.1.1.6 *Cell Volume*

Cell volumes, commonly called “biovolumes,” are used to roughly estimate algal biomass and provide an alternative way of expressing the quantity of algae present as opposed to unit or cell density. Cell volumes are determined by measuring the size of an algal cell and applying the measurements to the geometrical equation that best fits the algal cell shape. Cell volumes listed on the table have either been calculated by NCDWR staff, obtained through literature, or by consulting outside laboratories. The cell volumes listed are conservative and based upon the smallest measures or calculations so as to not overestimate biomass. The procedure to calculate the volume of an algal cell can be found in Section 5.6.

The accuracy of cell volume measurements is directly related to the number of measurements taken. If the cell volume for a particular taxon is not known, then a zero (0) should be listed on the table. Cell volumes should be updated as needed.

5.1.2 Taxonomic Revisions

The taxonomic reference list must reflect periodic changes in scientific nomenclature, thus it should be periodically reviewed and updated and when nomenclature has changed, biovolume information has been recalculated, or new taxa need to be added.

5.1.3 Adding Taxa

A taxon must be identified more than once and verified by a second qualified taxonomist before it can be placed on the table. Rare, obscure or tentative identifications, even with outside consultation, should not be placed on the table. Under no circumstances should a taxon be placed on the table without the consultation of other DWR taxonomists. The table should only contain taxa that are commonly found, easily referenced and relevant to the mission of the WSS.

5.2 Data entry

All data on samples and their analyses are entered into and stored in a Microsoft Access® database.

The final total from the database should be compared to the final total on the bench sheet. If the totals are not equal, then there is an error in either the bench sheet calculations or the entered data and any discrepancies must be rectified. Mark the box on the bench sheet labeled “Totals checked” when the two totals are equal.

5.3 Density calculations

Density can be expressed as unit density or cell density (Section 3.2). Unit densities are used in reports and cell densities are used to calculate biovolume estimates. Both density calculations are performed using the same equation. Only one calculation, the calculation for unit density, will be used in the example below. To calculate cell density, substitute cells for units in the equation.

Unit density is calculated for each individual taxon in the assemblage and for the entire assemblage. The unit density of each individual taxon is calculated first, and then the density of all the taxa are summed together to provide total density. The same procedure and equations are used to calculate individual and total cell density. Individual cell density is used to calculate sample biovolume.

5.3.1 Individual unit density calculation

The equation for individual unit density is:

$$\text{Individual Unit Density} = \frac{U}{A \times G \times \frac{C}{B}}$$

where:

U = total number of Taxon A units counted in all grids

A = area of the grid used

G = number of grids counted

C = volume of Utermöhl chamber used

B = total basal area of the Utermöhl chamber

Example calculation for the determination of Taxon A's individual unit density with an area of 0.003136 cm² and four grids counted:

$$\frac{18 \text{ units}}{0.003136 \text{ cm}^2 \times 4 \times \frac{10 \text{ ml}}{4.9087 \text{ cm}^2}} = \frac{18 \text{ units}}{0.02555 \text{ ml}} = 705 \frac{\text{units}}{\text{ml}}$$

Results: The unit density for Taxon A is 705 units/mL.

5.3.2 Total unit density

The equation for total unit density is:

$$\text{Total Unit Density} = \sum_{i=1}^n \frac{U}{A \times G \times \frac{C}{B}}$$

where:

U = total number of units counted in all grids

A = area of the grid used

G = number of grids counted

C = volume of Utermöhl chamber used

B = total basal area of the Utermöhl chamber

n = the total number of taxa

i = beginning with the 1st taxon

Example calculation for determination of Sample A's total unit density:

$$\frac{18 \text{ units}}{0.02555 \text{ ml}} + \frac{38 \text{ units}}{0.02555 \text{ ml}} + \frac{15 \text{ units}}{0.02555 \text{ ml}} + \frac{68 \text{ units}}{0.02555 \text{ ml}} + \frac{2 \text{ units}}{0.02555 \text{ ml}} =$$

$$705 \frac{\text{units}}{\text{ml}} + 1487 \frac{\text{units}}{\text{ml}} + 587 \frac{\text{units}}{\text{ml}} + 2661 \frac{\text{units}}{\text{ml}} + 78 \frac{\text{units}}{\text{ml}} =$$

$$\text{Total Unit Density} = 5519 \frac{\text{units}}{\text{ml}}$$

Results: The total unit density for sample A is 5519 units/mL; however, scientific convention calls for rounding or truncating values based on significant digit usage. In this example, which is the typical approach to unit density calculations, the result would include only two significant digits (5,500 units/mL).

5.4 Reference biovolume calculation

Biovolumes are calculated for each individual taxon (individual biovolume) in the assemblage and for the assemblage as a whole (total biovolume). These calculations are based upon the average biovolume (reference biovolume) of a taxon's cell and the taxon's cell density. The total biovolume of a sample is determined by adding the biovolumes of each individual taxon together.

A biovolume calculation worksheet documents how a particular taxon's biovolume was determined. Once determined, the taxon's biovolume is entered into the database and used as the reference biovolume for calculations.

5.4.1 Reference biovolume calculation worksheet

A reference biovolume calculation worksheet (Appendix D) must be completed on any new alga being added to the WSS taxa reference list or when reevaluating an existing biovolume. The worksheet must be stored in the filing cabinet designated for taxa information in the 4401 Algae Lab.

The worksheet should include:

- | | |
|--|---------------------------|
| ▪ Division | ▪ Authority |
| ▪ Family | ▪ Sample location |
| ▪ Genus | ▪ Date collected |
| ▪ Species (if applicable) | ▪ Variety (if applicable) |
| ▪ Digital images or drawings of the alga | |

Equations used to calculate biovolumes of different cell morphologies are in Appendix E.

5.4.2 Calculation of cell reference biovolume

All cells should be measured (μm) with an ocular reticule at 400X magnification or greater.

To determine the cell's reference biovolume:

1. Select the equation or set of equations that best fits the morphology of the algal cell from Appendix E.
2. Record the equation on the reference biovolume calculation worksheet.
3. Prepare a wet mount of the sample that contains the alga being evaluated.
4. Locate a specimen on the microscope slide.
5. Take the necessary measurements (μm) using the Whipple grid.
6. Specimen position can be changed by lightly tapping the cover slip with the tip of a pencil or pen. Practice is required to reposition the specimen correctly.
7. Record the measurements on the reference biovolume calculation worksheet.
8. Measure the cells of at least 15 specimens (preferably from more than one sample and during different seasons, since this calculation will ultimately represent an "ideal" or reference biovolume).
9. Determine the average size of a cell by adding all related measurements together then dividing by the number of measurements taken.

10. Use the average of these measurements in the selected equation to determine the reference biovolume of the cell.

Example of how to calculate Taxon A's reference biovolume where the cell is shaped like a rectangular box (Figure 1). Only 4 cells ($n = 4$) have been measured for the simplicity of this example (Table 4).

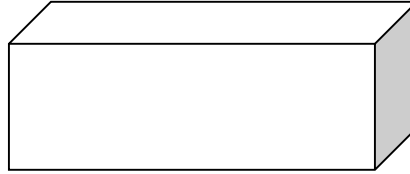


Figure 1. Example of a Cell Shape

Table 4. The length, width, and height measurements (μm) of 4 rectangular cells

Size	Length	Width	Height
cell 1	8	4	5
cell 2	10	6	6
cell 3	6	5	7
cell 4	8	6	5
totals	32	21	23
averages	8	5	6

Enter the average cells length, width and height into the equation for a rectangular box:

$$8 \mu\text{m} \times 5 \mu\text{m} \times 6 \mu\text{m} = 240 \mu\text{m}^3$$

Result: The reference biovolume of Taxon A is $240 \mu\text{m}^3$.

5.5 Calculation of an individual taxon's biovolume

To determine the biovolume of an individual taxon, multiply its reference cell biovolume (bv_{ref}) expressed in microns cubed (μm^3) by the number of cells found in 1 mL of the sample.

The equation for an individual taxon biovolume is:

$$\text{Individual Taxon Biovolume} = bv_{\text{ref}} (\mu\text{m}^3) \times \frac{\text{cells}}{\text{ml}}$$

Where:

bv_{ref} = reference biovolume of the alga

Example of the calculation for the individual biovolume of Taxon A:

$$240 \mu\text{m}^3 \times \frac{72 \text{ cells}}{\text{ml}} = 17,280 \frac{\mu\text{m}^3}{\text{ml}}$$

Results: The biovolume of taxon A is $17,280 \frac{\mu\text{m}^3}{\text{ml}}$

$$\text{Conversion of } \frac{\mu\text{m}^3}{\text{ml}} \text{ to } \frac{\text{mm}^3}{\text{m}^3}$$

The result of the equation for an individual taxon biovolume is expressed in microns cubed per milliliter ($\mu\text{m}^3/\text{mL}$) and must be converted to millimeters cubed per meters cubed (mm^3/m^3) to be consistent with historic data.

To convert $\mu\text{m}^3/\text{mL}$ to mm^3/m^3 :

- Multiply the individual taxon biovolume expressed in microns cubed per mL ($\mu\text{m}^3/\text{mL}$) by the number of millimeters cubed per microns cubed ($\text{mm}^3/(1 \times 10^9 \mu\text{m}^3)$).
- Multiply the individual taxon biovolume in one milliliter (mm^3/mL) by the number of milliliters in one liter (1000 mL/L).
- Multiply the individual taxon biovolume in one liter (mm^3/L) by the number of liters in one meter cubed (1000 L/ m^3).

The conversion equation is:

$$\text{bv} \frac{\text{mm}^3}{\text{m}^3} = \text{bv} \frac{\mu\text{m}^3}{\text{ml}} \times \frac{\text{mm}^3}{1 \times 10^9 \mu\text{m}^3} \times \frac{1000\text{ml}}{\text{L}} \times \frac{1000\text{L}}{\text{m}^3}$$

Example for converting Taxon A's biovolume from $\mu\text{m}^3/\text{mL}$ to mm^3/m^3 :

$$17,280 \frac{\mu\text{m}^3}{\text{ml}} \times \frac{\text{mm}^3}{1 \times 10^9 \mu\text{m}^3} \times \frac{1000\text{ml}}{\text{L}} \times \frac{1000\text{L}}{\text{m}^3} = 17.28 \frac{\text{mm}^3}{\text{m}^3}$$

Results: The biovolume for Taxon A is 17.28 mm^3/m^3

*Note: Dividing the $\text{bv} \frac{\mu\text{m}^3}{\text{ml}}$ by 1000 provides the same result.

$$\text{bv} \frac{\text{mm}^3}{\text{m}^3} = \text{bv} \frac{\mu\text{m}^3}{\text{ml}} \div 1000$$

5.6 Calculation of the total biovolume (BV)

The total biovolume of a sample is calculated by adding all the individual taxon biovolumes together.

The equation for the total biovolume is:

$$\text{BV} = \sum_{i=1}^n \text{bv} \frac{\mu\text{m}^3}{\text{ml}}$$

Where:

bv = biovolume of an individual taxon in 1 m^3 .

BV = total biovolume in 1 m^3 .

n = the total number of algae identified in the sample.

i = beginning with the 1st taxon.

Example of calculating the total biovolume for Sample A:

$$\textit{Taxon A} + \textit{Taxon B} + \textit{Taxon C} + \textit{Taxon D} = \textit{Total Biovolume}$$

$$17.28 \frac{\text{mm}^3}{\text{m}^3} + 14.78 \frac{\text{mm}^3}{\text{m}^3} + 36.89 \frac{\text{mm}^3}{\text{m}^3} + 8.79 \frac{\text{mm}^3}{\text{m}^3} = 77.74 \frac{\text{mm}^3}{\text{m}^3}$$

Results: The total biovolume in Sample A is = $78 \frac{\text{mm}^3}{\text{m}^3}$

5.7 Record Retention

Information pertaining to an episodic evaluation consists of, but is not limited to:

- Sample submittal form
- Bench sheet
- Summary report

Information pertaining to a routine evaluation consists of, but is not limited to:

- Study plan
- Bench sheet
- Summary report or final dataset/workbook

Related information, such as photos, maps, or news articles, are also archived along with the above items. The Division has modernized their records retention policy, including documents produced and used by the WSS. Hardcopies of episodic and routine evaluation records prior to 2010 are filed in individual folders in a file cabinet located in the lab. Subsequent years include both hardcopies, filed appropriately in the lab, and electronic sample submittal reports which are placed in folders per regional office on the WSS shared network drive. Study designs, reports, or dataset workbooks of routine evaluations are kept in folders organized by study.

6.0 QUALITY ASSURANCE

Current practices provide quality assurance on taxonomic identifications and data entry only.

6.1 Taxonomic Identifications

Staff expertise and a well-developed reference taxa list are the basis for accurate algal identifications over time. A reference taxa list maintains continuity between/among analysts and reduces problems associated with changes in nomenclature.

The current reference taxa list can be found in a unique table within the WSS algal database.

The individual taxa list should contain:

- Complete name and classification:
 - division
 - family
 - genus
 - species
 - variety
- Taxa code
- The reference(s) used to make the identification, along with the page number
- Size ranges
- Reference biovolume
- Habitat (freshwater or estuarine)
- Digital image (if available)

Only those taxa on the WSS reference taxa list may be entered into the database. A list of the primary reference materials used by the WSS can be found in the Reference section of this document.

6.2 Data Entry

Data are entered into the database on two occasions: 1) during the sample login procedure and 2) after analysis (bench sheet data). These data are checked for accuracy by a comparison of original records to entered data. Every entry must be reviewed by the lead taxonomist to check that the required data has been recorded correctly. The total number of cells and units must be tabulated and written on the bench sheet. These numbers must be compared to entered data and a check mark placed in the designated line to verify that the totals have been checked. Any discrepancies between original and entered data must be rectified (i.e., chamber volume, fields counted, dates collected and analyzed, and cell and unit calculations).

7.0 REFERENCES

- American Public Health Association (APHA). 1998. Standard Methods for the Examination of Water and Wastewater, 20th Edition. Washington, DC.
- Arar, E. and Gary Collins 1997. *In Vitro* Determination of Chlorophyll *a* and Pheophytin-*a* in Marine and Freshwater Algae by Fluorescence. EPA Method 445.0-1. Revision 1.2.
- Campbell, Peter H. 1973. *Studies on Brackish Water Phytoplankton*. University of North Carolina, School of Public Health. Sea Grant Publication. UNC-SG-73-07.
- North Carolina Department of Environmental Quality. Division of Water Resources (DWR). 2015. Fish Kill Response Procedures. Biological Assessment Unit. <http://www.ncnhp.org/web/wq/ess/fishkills>
- North Carolina Department of Environmental Quality. 2015. Sample Submission Guidance. Division of Water Resources (DWR). Laboratory Section. <http://deq.nc.gov/about/divisions/water-resources/water-resources-data/water-sciences-home-page/microbiology-inorganics-branch/sample-submission>
- North Carolina Department of Environment and Natural Resources. 2013. Standard Operating Procedures Manual: Physical and Chemical Monitoring. Intensive Survey Unit. http://portal.ncdenr.org/c/document_library/get_file?uuid=516f1b7b-fbb6-419f-83c8-0c981b2e1f78&groupId=38364
- North Carolina Department of Environment and Natural Resources. 2015. Quality Management Plan, Version 4.1. Original document submitted in 2011.
- Tomas, C., et al. 1997. *Identifying Marine Phytoplankton*. Academic Press, San Diego, CA.
- Vollenweider, R.A. 1974. A Manual for Measuring Primary Production in Aquatic Environments. IBP Handbook No. 12. F.A. Davis Company, Philadelphia
- Wehr, J. D., R. G. Sheath, Kociolek, P.J. (Eds). 2015. *Freshwater Algae of North America: Ecology and Classification*. Second Edition. Academic Press, San Diego, CA
- Wetzel, R.G. and G.E. Likens. 1991. *Limnological Analyses*, Second Edition Springer Verlag, New York, NY. 391 pp.
- Whitford, L.A. and G.J. Schumacher. 1984. *A Manual of Freshwater Algae*. Sparks Press, Raleigh, NC.

Appendix A. Field Report Forms

SAMPLE COLLECTION AND FIELD EVALUATION FORM FOR ALGAE, AQUATIC PLANTS, AND RELATED ORGANISMS

SAMPLE INFORMATION			
Sampler Name(s): _____		Agency: _____	
Date: _____	Time: _____	Station #: _____	
Waterbody/Address: _____		Basin: _____	County: _____
Latitude: _____		Longitude: _____	
HUC: _____			
Sample Type: <input type="checkbox"/> Filamentous Algae <input type="checkbox"/> Periphyton <input type="checkbox"/> Phytoplankton <input type="checkbox"/> Aquatic Plant <input type="checkbox"/> Unknown			
Collection Method: <input type="checkbox"/> Photic Zone <input type="checkbox"/> Grab <input type="checkbox"/> Scoop Attached: <input type="checkbox"/> Map <input type="checkbox"/> Photographs (PLEASE)			
Other Samples Collected: <input type="checkbox"/> Nutrients <input type="checkbox"/> Chlorophyll- <i>a</i> <input type="checkbox"/> Other: _____			
Algal Bloom Response? <input type="checkbox"/> Yes <input type="checkbox"/> No		Fish Kill Response? <input type="checkbox"/> Yes** <input type="checkbox"/> No	
**If yes, please fill out a NC DWR Fish Kill Field Investigation Form and submit with samples.			
ENVIRONMENTAL CONDITIONS			
Weather Conditions: _____			
Water Clarity: <input type="checkbox"/> Clear <input type="checkbox"/> Turbid <input type="checkbox"/> Tannic <input type="checkbox"/> Green <input type="checkbox"/> Other (Explain) _____			
Characteristics: <input type="checkbox"/> Filaments <input type="checkbox"/> Balls <input type="checkbox"/> Flecks <input type="checkbox"/> Surface Film <input type="checkbox"/> Other _____			
Algal Color: _____		Algal Abundance: _____ % Coverage	
Secchi Depth: _____ meters		Bottom Depth: _____ meters	

CHEMICAL AND PHYSICAL SAMPLE DATA						
Depth (m)	Cond (µS)	Temp (°C)	DO (mg/L)	DO (%sat)	pH (SU)	Salinity (ppt)
0.15 (surface)						
1.0						
2.0						
3.0						
4.0						

NOTES:

°C = degrees Celsius Cond = conductivity m = meter ppt = parts per trillion
 %sat = percent saturation DO = dissolved oxygen µS = ~~microSiemens~~ SU = standard units

SAMPLE INSTRUCTIONS

Sample collection should follow Standard Operating Procedures for the Collection and Analysis of Aquatic Algae (2016) and the Standard Operating Procedures Manual: Physical and Chemical Monitoring (2013). Sample quantities, handling requirements, and preservation methods are below. All samples should be kept on ice or in a refrigerator for storage and shipping.

Sample Type	Preservation Method	Quantity if Preserved	Quantity if Unpreserved
Filamentous Algae	Wet paper towel, plastic bag	N/A	2-3 golf-ball sized clumps
Periphyton	500 mL jar, 4mL Lugol's	50 milliliters of scrapings	2 small (4-inch) rocks/wood
Phytoplankton	500 mL jar, 2mL Lugol's	500 mL	500 mL
Aquatic Plant	Wet paper towel, plastic bag	N/A	3-5 stems, leaves, flowers, fruits

Send this form along with the sample and any supplemental information to the address included on the reverse. Copies of this and other sample collection forms can be obtained by calling (919) 743-8400 or visiting www.deq.nc.gov.

NC DWR Fish Kill Field Investigation Form

Send To: DWR Environmental Sciences Section Attn: Mark Hale or Elizabeth Fensin
1621 Mail Service Center, Raleigh, NC 27699-1621 (919) 743-8400 Fax (919) 743-8517
e-mail: mark.hale@ncdwr.gov elizabeth.fensin@ncdwr.gov

Investigators:		Kill Number (ESS use only):	
Investigation Date and Time:		Manhours Required:	
Investigators:			
Organization:		Regional Office:	
Address:		Phone:	
Reporting Party:	Address:	Phone:	
Coinvestigators:	Address:	Phone:	

Kill Event Location:

Waterbody:	Station:	Subbasin:
County:	Nearest Town/Landmark:	
Tributaries or other waters affected:		
Latitude:	Longitude:	

Attach map describing area of event and provide coordinates

Complete For Fish Kill and Disease Events

Date Event Began (First reported):	Time:
At time of investigation, kill event is : in progress <input type="checkbox"/> completed: <input type="checkbox"/>	
Area covered by kill: River (miles):	Lake / Estuary (acres, square miles):
Event Duration: Days:3	Hours:

Finfish Species Affected:

Species:	Size Range:	Approx Number:
In Distress / Dying <input type="checkbox"/> Dead <input type="checkbox"/> Decayed <input type="checkbox"/>	Percent observed with sores or lesions:	
Species:	Size Range:	Approx Number:
In Distress / Dying <input type="checkbox"/> Dead <input type="checkbox"/> Decayed <input type="checkbox"/>	Percent observed with sores or lesions:	
Species:	Size Range:	Approx Number:
In Distress / Dying <input type="checkbox"/> Dead <input type="checkbox"/> Decayed <input type="checkbox"/>	Percent observed with sores or lesions:	
Species:	Size Range:	Approx Number:
In Distress / Dying <input type="checkbox"/> Dead <input type="checkbox"/> Decayed <input type="checkbox"/>	Percent observed with sores or lesions:	

Other Organisms Affected:

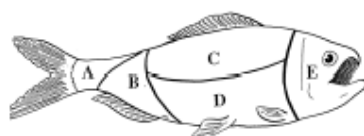
Total Finfish Mortality:	Total Mortality of Other Organisms:
--------------------------	-------------------------------------

Fish Disease Observations:

Lesions/Sores <input type="checkbox"/> Injuries <input type="checkbox"/> Flared Gills <input type="checkbox"/> Excessive mucus <input type="checkbox"/> Tumors <input type="checkbox"/> Visible Parasites <input type="checkbox"/>
Gasping <input type="checkbox"/> Loss of equilibrium <input type="checkbox"/> Erratic behavior <input type="checkbox"/> Attempts to leave water <input type="checkbox"/> Lethargy <input type="checkbox"/>
Convulsions <input type="checkbox"/> Other <input type="checkbox"/> Describe:

General Lesion and Sore Observations:

Size(cm):	Location (view diagrams below):
Appearance/ Comments:	



Physical Observations and Water Quality Measurements:

Station:	Station No.:	Time:
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Cloud Cover:	Seas:	Air Temp:	Precip:
Wind Direction:	Wind Speed:	Secchi Depth:	
Bottom Depth:	Prior Weather (3-4 days):		
Outfalls Present: Y <input type="checkbox"/> N <input type="checkbox"/> Describe:			
Spills in area ? Y <input type="checkbox"/> N <input type="checkbox"/> Describe:			
Other Activity:			

Depth (meters)	Dissolved Oxygen(mg/L)	% Saturation	pH	Temperature (°C)	Conductivity	Salinity (ppt)
Surface						
1.0						
2.0						
3.0						
4.0						
5.0						

Algal Bloom: Fill out this section if bloom occurs in conjunction with fish kill or disease. If bloom occurs without affected fish, fill out Algal Bloom Report form only and send to Division of Water Quality ESS Attn: Ecosystems Unit Staff

Visual characteristics:

<input type="checkbox"/> Discolored Water	Describe:
<input type="checkbox"/> Flecks, <input type="checkbox"/> Balls, <input type="checkbox"/> Filaments	
<input type="checkbox"/> Surface Film	
<input type="checkbox"/> Other	

Phytoplankton Samples Sent to ESS? Yes ☐ No ☐ *Phytoplankton Sample Quantity: One 500ml polyethylene container preserved with 2-3 ml Lugols solution and one 500ml polyethylene container unpreserved. Send to DWQ Laboratory Section marked Attn: Ecosystem Unit Staff.*

Biological and Chemical Samples Collected During Investigation:

Fish (Describe):	Iced, Preserved ?
Station:	Sample No.:
Contact for Results:	Phone:
Algae/Plants (Describe):	Iced, Preserved ?
Station:	Sample No.:
Contact for Results:	Phone:
Other (Describe):	Iced, Preserved ?
Station:	Sample No.:
Contact for Results:	Phone:

Chemical Samples: Metals <input type="checkbox"/> Pesticides <input type="checkbox"/> Nutrients <input type="checkbox"/> Chlor A <input type="checkbox"/> Other <input type="checkbox"/>	Describe:
Station:	Sample No.:
Contact for Results:	Phone:

Photographs and/or videos taken? Y ☐ N ☐

Where on file? Name:	Phone:
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Additional Comments, Observations, Conclusions, etc. (attach additional sheet if necessary):

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Appendix B. Modified Lugol's Solution Preparation

A modified Lugol's solution (Vollenweider 1974) is used to preserve phytoplankton and periphyton samples. The modification is glycerin, which is added to the traditional Lugol's solution to help prevent loss of flagella.

The solution should be prepared in a fume hood. The preparer should wear gloves, goggles, and a lab coat.

The following amounts of chemicals are used to make about 1 L of the solution:

- 40 g Iodine
- 80 g Potassium Iodide
- 80 mL Glacial Acetic Acid
- 800 mL Distilled Water
- 50 mL Glycerin
- 50 mL 95% Ethyl Alcohol

To prepare the solution:

1. Pour 80 mL of Glacial Acetic Acid into 800 mL of Distilled Water
2. Add 80 g of potassium iodide
3. Add 40 g Iodine
4. Add 50 mL Glycerin
5. Add 50 mL of 95% ethyl alcohol
6. Mix thoroughly

****Caution:** Iodine will seep through plastic containers and leave stains on shelves and floors.

Lugol's solution is photosensitive. It must be stored in an opaque container in a cool, dark place.

Appendix C. Phytoplankton Analysis Bench Sheet

Phytoplankton Analysis Bench Sheet			
Sample ID	Chem Lab ID	Visual Scan	
Date Collected	Date Analyzed	Analyst	Total Cells
Waterbody	Grids Counted	Total Units	
Site	Grid Size	Date Entered	
QA ID	Chamber Volume 5 10 20		

	Taxon		NCDWRTSN	Cells	Units
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					
Totals					

Appendix D. Example of Biovolume Calculation Worksheet/New Taxa Record Form

Biovolume Calculation Worksheet/New Taxa Record Taxa code _____

Pg ____ of ____

Division _____

Class _____

Order _____

Family _____

Genus _____

Species _____

Variety _____

Authority/date _____

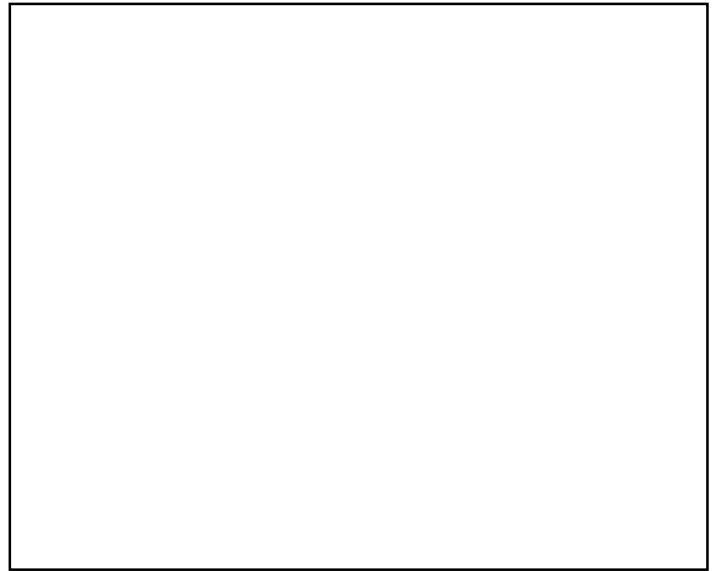
Reference _____

Habitat _____

Size Ranges _____

Biovolume _____

Picture or Drawing



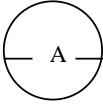
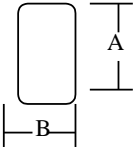
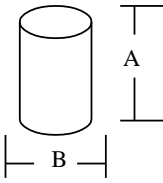
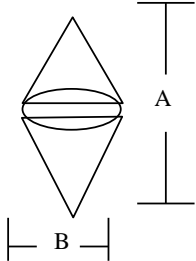
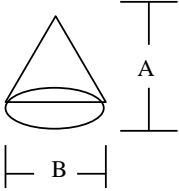
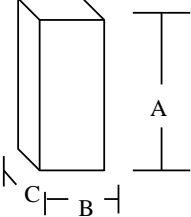
Equation/s used for biovolume _____

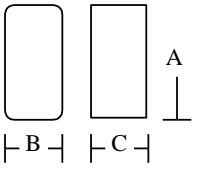
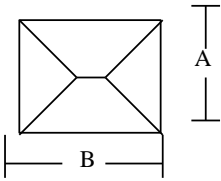
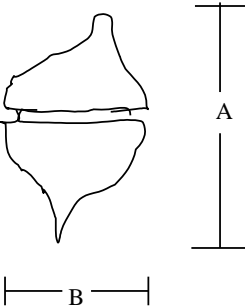
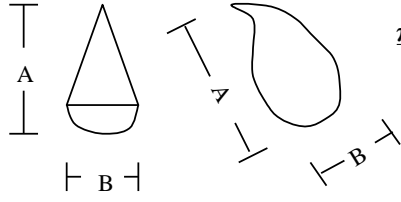
Description

Biovolume calculation information:

<u>Waterbody</u>	<u>Date</u>	<u>Cell#/unit</u>	<u>Length</u>	<u>Width</u>	<u>Height</u>	<u>Biovolume</u>
AVERAGES	_____					

Appendix E. Equations for Biovolume (modified from Wetzel & Likens 1991)

Shape	Diagram	Formula	Representative species
Sphere		$\pi A^3/6$	<i>Sphaerocystis schroeteri</i>
Ellipsoid		$\pi AB^2/6$	<i>Scenedesmus bijuga</i> <i>Cryptomonas</i> <i>Euglena</i>
Rod		$\pi AB^2/4$	<i>Melosira granulata</i> <i>Cyclotella</i> <i>Asterionella</i>
Two cones		$\pi AB^2/12$	<i>Ankistrodesmus falcatus</i>
One cone		$\pi AB^2/12$	(Horn of <i>Ceratium</i>)
Rectangular box		ABC	

Shape	Diagram	Formula	Representative species
Irregular		$BC(A - B + \frac{\pi}{4}B)$	<i>Achnanthes</i> B: Valve view C: girdle view
Irregular		$A^3/4$	<i>Crucigenia tetrapedia</i>
Irregular		$\pi AB^2/9$	<i>Peridinium</i>
Ellipsoid/cone	 <p>(1/2 ellipsoid + 1 cone)</p>	$\frac{\pi B^2(A + B/2)}{12}$	<i>Rhodomonas minuta</i> <i>Gymnodinium</i> <i>Synura</i>